Article

Characterization of MPL-mutated myeloid neoplasms: a review of 224 MPL+ cases

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Abstract: Mutations within the myeloproliferative leukemia virus (*MPL*) gene have been identified in some myeloid neoplasms; however, myeloid neoplasms with MPL mutations (MPL+) have not been well characterized. This study investigated the disease distribution, morphology, cytogenetic abnormalities, and select clinical features of MPL+ myeloid neoplasms in 224 cases. Results showed that MPL mutations occur in myeloid neoplasms including primary myelofibrosis, essential thrombocytosis, myeloproliferative neoplasm, unclassifiable, polycythemia vera, refractory anemia with ring sideroblasts and marked thrombocytosis, and myeloid neoplasms associated with PDGFB rearrangement. Compared to cases with no MPL mutations (MPL-), MPL+ cases are associated with lower hemoglobin, lower WBC counts, older age, higher reticulin scores, and lower bone marrow cellularity. Compared to MPL-/JAK2+ (with *JAK2* mutations), MPL+/JAK2- (with no *JAK2* mutation) cases are associated with lower hemoglobin, lower WBC counts, lower bone marrow cellularity, but no significant differences in reticulin scores or patient age. Compared to MPL-/JAK2-, MPL+/JAK2- cases show significantly higher reticulin scores, but no statistical differences in hemoglobin, WBC counts, patient age, or bone marrow cellularity. MPL and JAK2 V617F mutations can coexist (7.9%); however, MPL mutations and BCR/ABL appear mutually exclusive. Overall incidence of cytogenetic abnormalities was not significantly different in cases with or without MPL mutations. In this study, MPL positivity alone does not appear to result in a distinct entity.

Keywords: BCR/ABL, JAK2, MPL, myeloid neoplasms.

Introduction

The discovery of BCR/ABL offered the first insight into the molecular basis of myeloproliferative neoplasms (MPNs), and ultimately led to the development of targeted therapy for chronic myelogenous leukemia (CML). The later discovery of mutations in JAK2 and MPL provided further understanding of the pathogenesis of BCR/ABL negative myeloid neoplasms [1–7]. Both JAK2 and MPL mutations induce constitutive, cytokine-independent activation of the JAK-STAT pathway [2]. The myeloproliferative leukemia virus (MPL) gene encodes the thrombopoietin receptor, and thus plays a key role in megakaryocyte growth and survival. Mutations within exon 10 targeting the transmembrane domain of the MPL receptor result in ligand-independent receptor activation. MPL mutations have been reported in 5% to 10% of patients with primary myelofibrosis (PMF)

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and 1% of patients with essential thrombocytosis (ET) [1, 2]. Reports regarding the occurrence of MPL mutations in other myeloid neoplasms, and the clinical and laboratory characteristics of MPL+ cases have been conflicting [5, 8–12], likely due to the relatively low incidence of MPL+ cases, small sample sizes, and challenges in diagnosis and classification of these entities. To date, a large scale, systematic histopathologic study of MPL+ myeloid neoplasms has not been performed. With the advantage of our large case volume, we investigated disease distribution, morphologic characteristics, cytogenetics, and select clinical features of MPL+ myeloid neoplasms.

Materials and Methods

After receiving IRB approval, we identified 224 MPL+ (K- and L-allele mutations) myeloid neoplasms in blood or bone marrow from our case files from January 2008 to February 2012 and randomly selected 300 MPL-, BCR/ABL- myeloid neoplasms (including refractory anemia with ring sideroblasts and marked thrombocytosis [RARS-T]) as controls. Some cases were excluded from parts of the analysis when clinical data indicated conditions that might influence morphology, CBC data, or cytogenetic findings (e.g., recent chemotherapy, concurrent nonhematopoietic neoplasm, or treatment such as hydroxyurea or phlebotomy).

Overall, 112 MPL+ cases and 128 MPL- control cases qualified for morphologic evaluation and confirmation of disease classification. Bone marrow cellularity, reticulin scores, bone marrow blast count, and peripheral blood blast count were evaluated. Diagnosis and disease classification were based on the 2008 WHO classification [13]. Original diagnoses were confirmed by at least one pathologist from a panel of four investigating pathologists. In cases of a discrepancy between the primary pathologist and the reviewing pathologist, the case was reviewed by a panel of 2 to 3 pathologists, who developed a consensus opinion.

Clinical parameters including patient age, gender, hemoglobin level, WBC count, and platelet count were collected and compared between the study groups.

Frequency of concomitant JAK2 V617F (by polymerase chain reaction [PCR]) or BCR/ABL positivity (by karyotype, FISH or PCR) was also evaluated. In addition, 233 BCR/ABL+ MPN cases were identified to evaluate the frequency of MPL mutations within that group.

Molecular testing

DNA isolation was performed at Genoptix, Inc. The presence of the JAK2 V617F (1849G>T) genotype, MPL W515L (TGG>TTG), and MPL W515K (TGG>AAG) genotypes was determined with PCR via Cleavase-based allele-specific signal amplification. BCR/ABL translocation was detected using quantitative reverse transcriptase (RT)-PCR that detects fusion transcripts resulting from the breakpoints b3a2, b2a2, b2a3, b3a3 and e1a2 occurring in the major breakpoint cluster region (M-bcr) and minor breakpoint cluster region (m-bcr).

Karyotype and FISH

Tissue cultures were performed at Genoptix, Inc. Chromosome analysis and FISH technical components were also performed at Genoptix, Inc. or at cooperative laboratories. Of all the MPL+ and control group cases, 167 MPL+ cases and 233 MPL- cases with karyotyping, FISH, or both were available for cytogenetic analysis.

Statistical analysis

Data statistical analyses were based on parameters collected at the time of initial diagnosis. Conventional U-test and χ^2-statistics were performed using Graphpad Prism software. All P-values were two tailed and statistical significance was set at the level of $P<0.05$.

Table 1: Disease distribution of MPL+ and MPL- cases.

Disease distribution	JAK2+/MPL+ (n=4)	JAK2-/MPL+ (n=109)	JAK2+/MPL- (n=82)	JAK2-/MPL- (n=46)	Total MPL+ (n=114)	Total MPL- (n=128)
PMF	1	58	33	18	59 (51.8%)	51 (40.0%)
ET	1	35	28	15	36(31.6%)	43 (33.6%)
PV	1	0	8	0	1 (0.9%)	8 (6.3%)
MPN, U	1	11	10	9	12 (10.5%)	19 (14.9%)
RARS-T	0	4	3	4	4 (3.5%)	7 (5.5%)
PDGFB	0	1	0	0	1 (0.9%)	0

Results

The disease distribution of the 114 MPL+ cases with morphologic evaluation and confirmed diagnosis is shown in Table 1. These include 59 cases of PMF (51.8%), 36 cases of ET (31.6%), 12 cases of MPN, unclassifiable (MPN, U) (10.5%), 4 cases of RARS-T (3.5%), one case of polycythemia vera (PV) (0.9%), and one case of myeloid neoplasms associated with *PDGFB* rearrangement.

Of the 224 MPL+ cases, 35 cases were positive for K allele (15.6%) and 189 cases were positive for L allele (84.4%). There were no cases with both the K and L alleles. Of the MPL L-allele positive cases, 16 were positive for JAK2 V617F mutation (8.5%, n=189). No cases with the MPL K allele were JAK2 positive.

A comparison of the morphologic findings between MPL+ and MPL- cases are shown in Table 2. MPL+ cases showed higher reticulin scores (1.546 $\pm$ 0.1112, n=108, versus 1.168 $\pm$ 0.1033, n=125, P=0.0134) and lower bone marrow cellularity than MPL- cases (49.56 $\pm$ 1.636, n=112, versus 57.50 $\pm$ 1.785, n=126, P=0.0013). There were no significant differences in bone marrow blast counts or peripheral blood blast counts between the two groups. Further analysis showed that the MPL+/JAK2- (n =103) cases displayed no significant difference in reticulin scores in comparison to MPL-/JAK2+ cases (n =81; P=0.0572). However, MPL+/JAK2- cases did show significantly higher reticulin scores than MPL-/JAK2- cases (P=0.0310). In addition, MPL+/JAK2- cases showed significantly lower cellularity than MPL-/JAK2+ cases (P<0.0002), but there was no statistical difference in cellularity compared to MPL-/JAK2- cases (P=0.2092). The MPL+/JAK2+ group was not included in this portion of the analysis due to the small number of cases identified.

The association between MPL mutation status and clinical variables is shown in Table 3. MPL+ cases showed a slight female predominance compared to the MPL- cases; although this finding was not statistically significant (P=0.3235). Patients with MPL mutations showed lower hemoglobin levels (12.02 $\pm$ 0.1729, n=166, versus 13.01 $\pm$ 0.1739, n=195, P<0.0001), lower WBC counts (9.078 $\pm$ 0.4348, n=168, versus 12.15 $\pm$ 0.5289, n=197, P<0.0001), and older age (72.40 $\pm$ 0.7834, n=224, versus 68.97 $\pm$ 1.032, n=195, P=0.0076) than those of patients with unmutated MPL. A majority of patients with MPL mutations had thrombocytosis (128/168, 76.2%). Normal platelet counts and thrombocytopenia were less frequent (29/168, 17.3%, and 11/168, 6.5%, respectively), but there was no significant statistical difference in platelet counts between MPL+ and MPL- groups (631.2 $\pm$ 26.16, n=166, versus 673.8 $\pm$ 22.60, n=195, P=0.2157).

Further analyses (Table 4) showed that in comparison to MPL-/JAK2- cases, those with MPL+/JAK2- showed a trend towards lower hemoglobin levels and lower WBC counts, but these did not reach statistical significance (P=0.6517 and .0752, respectively). There was also no statistical difference in patient age between these two groups (P=0.1468). The MPL+/JAK+ group was not included in this portion of the analysis due to the small number of

Table 2: Morphologic characteristics of MPL+ and MPL- cases.

Morphologic parameters	MPL+	MPL-	*P*
Cellularity, % (±SE), (n)	49.56 ± 1.636 (112)	57.50 ±1.785 (126)	0.0013
Average reticulin score (0-3), (±SE) (n)	1.546 ± 0.1112 (108)	1.168 ± 0.1033 (125)	0.0134
Bone marrow blast count (<5%), % (n)	100 (112/112)	100 (128/128)	
Bone marrow last count (>5%), % (n)	0 (0/112)	0 (0/128)	
Peripheral blood blast count (<1%), % (n)	85 (56/66)	87 (66/76)	
Peripheral blood blast count (1-5%), % (n)	15 (10/66)	13 (10/76)	

cases identified.

Cytogenetic results are shown in Table 5. Of the 167 MPL+ cases and 233 MPL- cases that were qualified for cytogenetic analysis, cytogenetic abnormalities were found in 26 MPL+ cases (15.6%), including 6 cases with 13q- (3.6%), 9 cases with 20q- (5.4%), 2 cases with 5q- (1.2%), 2 cases with +8 (1.2%), 2 cases with 7q-/-7 (1.2%), and 2 cases with -Y (1.2%). Five cases showed two cytogenetic abnormalities. Other abnormalities [including 9q-, +1q, PDGFB, t(1;3), 18p-, t(12;14), and t(6;12)] were only found in single cases. There was no significant difference in overall incidence of cytogenetic abnormalities in MPL+ cases compared to MPL- cases. Of note, the incidence of 13q deletion appeared higher in MPL+/JAK2- group than MPL-/JAK2+ group but was not statistically significant (*P*=0.0659).

The possibility of concomitant BCR/ABL translocation and MPL or JAK2 mutations were also investigated; 262 CML cases with positive BCR/ABL translocation were separately identified. Two JAK2+ cases were found in 239 CML cases. None of the 262 CML cases were positive for MPL mutations. In addition, none of the 224 MPL+ cases were positive for BCR/ABL.

Discussion

Originally, MPL mutations were only detected in PMF and ET patients. Subsequently, MPL positivity was identified in PV, MDS with 5q-, AML, and RARS-T patients [8, 9, 11]. Similar to those previous studies, we also found MPL mutations in MPN, U, PV, RARS-T, ET, and PMF. However, we also identified MPL positivity in one case of PDGFB. Although MPL positivity in MDS with 5q- was previously reported by others, we found no such cases. Within the study cohort, there were four PV or PV-like cases that were positive for both JAK2 and MPL mutations. Two of them satisfied the WHO criteria for PV. The third one was a peripheral blood sample with hemoglobin level of 22.2 g/dL and no follow up samples, or information regarding serum erythropoietin. The fourth case was also a peripheral blood sample with possible iron deficient PV, with hemoglobin of 10.0 g/dL and MCV of 72, but no follow-up samples.

Mutations of MPL can be seen in patients with thrombocytosis (128/168, 76.2%), normal platelet count (29/168, 17.3%) or thrombocytopenia (11/168, 6.5%). In our daily practice, MPL mutations are usually tested when thrombocytosis is present and

Table 3: Clinical parameters of MPL+ and MPL- cases.

Clinical parameters	MPL+	MPL-	*P*
Sex (male/female ratio) (n)	0.66:1, 89/135 (224)	0.81:1, 88/109 (197)	0.3235
Mean age, years (±SE) (n)	72.4 ± 0.7834 (224)	68.97 ± 1.032 (195)	0.0076
Mean WBC count, x109/L (±SE) (n)	9.078 ± 0.4348 (168)	12.15 ± 0.5289 (197)	0.0001
Mean hemoglobin, g/dL (±SE) (n)	12.02 ± 0.1729 (166)	13.01 ± 0.1739 (195)	0.0001
Mean platelet count, x109/L (±SE) (n)	631.2 ± 26.16 (166)	673.8 ± 22.60 (195)	0.2157

Table 4: Effect of JAK2 and MPL mutations on clinical and morphologic parameters.

	P	
	MPL+/JAK2- vs MPL-/JAK2+	**MPL+/JAK2- vs MPL-/JAK2-**
Hemoglobin	<0.0001	0.6517
WBC count	<0.0001	0.0752
Platelet count	0.9478	0.1801
Age	0.0670	0.1468
Sex	0.4931	0.8737
Reticulin score	0.0572	0.0310
Cellularity	0.0002	0.2092

an MPN is suspected. Based on results of this study, we suggest that MPL mutation testing should be considered when MPN or MDS/MPN is suspected regardless of the patient's platelet count, especially when the JAK2 mutation result is negative.

In addition, MPL mutations have been reported in association with other mutations/cytogenetic abnormalities including JAK2 mutation and BCR/ABL translocation [14]. In this study, MPL and JAK2 double mutations occurred (7.9%); JAK2 mutation and BCR/ABL translocation also coexisted in a small number of cases (2/239). However, we did not identify any cases with both BCR/ABL and MPL mutations, suggesting that MPL mutations and BCR/ABL translocation may be mutually exclusive. An even larger cohort may be needed to exclude the possibility of very low incidence of concomitant MPL mutations and BCR/ABL translocation.

The morphologic analyses of this study show that MPL+ cases display higher reticulin scores and lower bone marrow cellularity than MPL- cases. Further stratification shows that MPL+/JAK2- cases display significantly higher reticulin scores than MPL-/JAK2- cases but no significant difference in reticulin scores compared to MPL-/JAK2+ cases. In addition, MPL+/JAK2- cases show significantly lower cellularity than MPL-/JAK2+ cases (P<0.0002), but no significant difference than MPL-/JAK2- cases (P=0.2092). Finally, there are no significant differences in bone marrow or peripheral blood blast counts between MPL+ and MPL- cases. It appears that the differences between MPL positive and negative cases are mainly due to the influence of the JAK2 mutation.

In this study, the MPL+ cases showed a similar overall incidence of cytogenetic abnormalities to that of previous studies [3]. The most common cytogenetic abnormalities found in the MPL+ cases in this study were 13q-, 20q-, 5q-, and +8. Of note, the incidence of 13q deletion appeared higher in MPL+/JAK2- group (6/153) than in the MPL-/JAK2+ group (1/155) but was statistically insignificant (P=0.0659). Evaluation of a larger cohort may show statistical significance.

In this study, MPL mutation status provided diagnostic value in addition to JAK2 mutation testing, cytogenetic analysis, and other laboratory studies. We conclude that MPL mutation testing should be performed in patients with clinical or morphologic features of MPNs especially those with negative JAK2 mutation test results. The presence of MPL mutation positivity may help establish a diagnosis of MPN or RARS-T. In cases with overlapping features between MDS and MPN, MPL mutation positivity may aid in disease categorization.

Although not identified in this study, the possibility of MPL mutations in other myeloid neoplasms such as chronic myelomonocytic leukemia (CMML), chronic neutrophilic leukemia (CNL), chronic eosinophilic leukemia (CEL), myeloid neoplasms associated with PDGFRA rearrangement (PDGFRA), stem cell leukemia/lymphoma syndrome with 8p11 (FGFR1) abnormalities (FGFR1), or MDS cannot be excluded. In myeloid neoplasms lacking thrombocytosis, such as CMML, MDS, or the more rare disorders of CNL, CEL, and PDGFRA, MPL testing may not be ordered, and thus the incidence of MPL positivity in these disorders may not be represented in this study.

Vannucchi *et al* [4] reported patients with MPL+ (n=30) showed lower hemoglobin levels, higher platelet counts and reduced total cellularity in ET patients compared to MPL-/JAK2 V617F+ cases. A study by Beer *et al* [3] reported that patients with

Table 5: Cytogenetic findings in MPL+ and MPL- cases.

Cytogenetic abnormalities	13q-	20q-	5q-	+8	7q-/-7	-Y	+1q	i(17q)	+9	Complex (≥3)	Other	Total
MPL+ (%) n=167	6 (3.6%)	9 (5.4%)	2 (1.2%)	2 (1.2%)	2 (1.2%)	2 (1.2%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	7 (4.2%)	26 (15.6%)
MPL- (%) n=233	4 (1.7%)	14 (6.0%)	4 (1.7%)	7 (3.0%)	4 (1.7%)	3 (1.3%)	3 (1.3%)	2 (1.0%)	2 (1.0%)	3 (1.3%)	5 (2.2%)	41 (17.6%)

MPL mutations (n=44) were significantly older and showed lower cellularity than those who were JAK2 V617F negative, and displayed a lower hemoglobin level and higher platelet count than those who were JAK2 V617F positive. A study by Guglielmelli *et al* [15] showed that the presence of an MPL mutation in patients with PMF (n=18) was associated with female gender, older age, lower hemoglobin level, and a higher likelihood of becoming transfusion-dependent. The discrepancies between this study and these previously published studies may be due to the relatively small numbers of cases investigated by other groups, differences in patient populations, and/or difficulties in the diagnosis of some of the MPNs, especially those cases which are post-PV, post-ET, in the fibrotic phase of PMF, or MPN, U since the morphologic findings overlap. In addition, some of the previous studies lack morphologic confirmation of the primary diagnosis by other pathologists; as a result, disease classification may be less accurate. Furthermore, in our reference lab setting, some of the clinical information is not available and clinical follow up is difficult. Thus, some clinical parameters such as disease survival, disease transformation, and treatment response are not included in this study.

In this study, no cases of concurrent mutations of K and L alleles were identified. However, the possibility of multi-mutations in one patient cannot be excluded from this study since we only tested for MPL W515L and MPL W515K mutations. Other MPL mutations reported by other investigators [6, 16, 17] were not included in this study.

In summary, MPL mutations can be detected in a variety of myeloid neoplasms including PMF, ET, MPN, U, PV, RARS-T, and PDGFB. Particularly amongst BCR/ABL and JAK2 negative cases, MPL provides diagnostic value that may help diagnose a myeloid neoplasm and can help distinguish between MPNs and MDS/MPNs.

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Author Contributions

K.L. conceived of and designed the study; K.L., Z.W., M.I., G.X., and P.P. collected and assembled the data; K.L., Z.W., and M.I. analyzed and interpreted the data; K.L. and K.J reviewed the data and served as the primary writers; M.I., B.D., and J.J. reviewed the data analysis and contributed to manuscript editing; and all authors provided final approval of the manuscript.

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References

1. Pikman Y, Lee BH, Mercher T, et al. MPLW515L is a novel somatic activating mutation in myelofibrosis with myeloid metaplasia. PLoS Med. 2006;3(7):e270.
2. Pardanani AD, Levine RL, Lasho T, et al. MPL515 mutations in myeloproliferative and other myeloid disorders: a study of 1182 patients. Blood. 2006;108(10):3472-3476.

3. Beer PA, Campbell PJ, Scott LM, et al. MPL mutations in myeloproliferative disorders: analysis of the PT-1 cohort. Blood. 2008;112(1):141-149.
4. Vannucchi AM, Antonioli E, Guglielmelli P, et al. Characteristics and clinical correlates of MPL 515W4L/K mutation in essential thrombocythemia. Blood. 2008;112(3):844-847.
5. Xu W, Li JY, Xia J, Zhang SJ, Fan L, Qiao C. MPL W515L mutation in Chinese patients with myeloproliferative diseases. Leuk Lymphoma. 2008;49(5):955-958.
6. Pardanani A, Guglielmelli P, Lasho TL, et al. Primary myelofibrosis with or without mutant MPL: comparison of survival and clinical features involving 603 patients. Leukemia 2011;25(12):1834-1839.
7. Vakil E, Tefferi A. BCR-ABL1-negative myeloproliferative neoplasms: a review of molecular biology, diagnosis, and treatment. Clin Lymphoma Myeloma Leuk. 2011;11(suppl 1):S37-S45.
8. Hussein K, Bock O, Theophile K, et al. MPLW515L mutation in acute megakaryoblastic leukaemia. Leukemia. 2009;23(5):852-855.
9. Patnaik MM, Lasho TL, Finke CM, et al. WHO-defined 'myelodysplastic syndrome with isolated del(5q)' in 88 consecutive patients: survival data, leukemic transformation rates and prevalence of JAK2, MPL and IDH mutations. Leukemia. 2010;24(7):1283-1289.
10. Gurevich I, Luthra R, Konoplev SN, Yin, CC, Medeiros LJ, Lin P. Refractory anemia with ring sideroblasts associated with marked thrombocytosis: a mixed group exhibiting a spectrum of morphologic findings. Am J Clin Pathol. 2011;135(3):398-403.
11. Malcovati L, Dell Porta MG, Pietra D, et al. Molecular and clinical features of refractory anemia with ringed sideroblasts associated with marked thrombocytosis. Blood. 2009;114(17):3538-3545.
12. Pardanani A, Lasho TL, Finke CM, Tefferi A. Infrequent occurrence of MPL exon 10 mutations in polycythemia vera and post-polycythemia vera myelofibrosis. Am J Hematol. 2011;86(8):701-702.
13. Swerdlow SH, Campo E, Harris NL, et al. WHO classification of tumours of haematopoietic and lymphoid tissues, 4th edition. Lyon:IARC;2008.
14. Hussein K, Bock O, Theophile K, et al. Chronic myeloproliferative diseases with concurrent BCR-ABL junction and JAK2V617F mutation. Leukemia. 2008;22(5):1059-1062.
15. Guglielmelli P, Pancrazzi A, Bergamaschi G, et al. Anaemia characterises patients with myelofibrosis harbouring Mpl mutation. Br J Haematol. 2007;137(3):244-247.
16. Schnittger S, Haferlach C, Beelen DW, et al. Detection of three different MPLW515 mutations in 10.1% of all JAK2 V617 unmutated ET and 9.3% of all JAK2 V617F unmutated OMF: a study of 387 patients [abstract]. Blood. 2007;110. Abstract 2546.
17. Vannucchi AM, Antonioli E, Pancrazzi A, et al. The clinical phenotype of patients with essential thrombocythemia harboring MPL 515W4L/K mutation [abstract]. Blood. 2007;110. Abstract 678.